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Lipopeptide families at the interface between pathogenic and beneficial *Pseudomonas*-plant interactions

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ABSTRACT

Lipopeptides (LPs) are a prominent class of molecules among the steadily growing spectrum of specialized metabolites retrieved from *Pseudomonas*, in particular soil-dwelling and plant-associated isolates. Among the multiple LP families, pioneering research focussed on phytotoxic and antimicrobial cyclic lipopeptides (CLPs) of the ubiquitous plant pathogen *Pseudomonas syringae* (syringomycin and syringopeptin). Their non-ribosomal peptide synthetases (NRPSs) are embedded in biosynthetic gene clusters (BGCs) that are tightly co-clustered on a pathogenicity island. Other members of the *P. syringae* group (*Pseudomonas cichorii*) and some species of the *Pseudomonas asplenii* group and *Pseudomonas fluorescens* complex have adopted these biosynthetic strategies to co-produce their own mycin and peptin variants, in some strains supplemented with an analogue of the *P. syringae* linear LP (LLP), syringafactin. This capacity is not confined to phytopathogens but also occurs in some biocontrol strains, which indicates that these LP families not solely function as general virulence factors. We address this issue by scrutinizing the structural diversity and bioactivities of LPs from the mycin, peptin, and factin families in a phylogenetic and evolutionary perspective. BGC functional organization (including associated regulatory and transport genes) and NRPS modular architectures in known and candidate LP producers were assessed by genome mining.

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1. Introduction

Pseudomonas inhabit diverse environments and many species are thriving in the rhizosphere and phyllosphere of plants (Weller 2007; Silby et al. 2011). Most plant-associated *Pseudomonas* belong to the *P. fluorescens* lineage, which is composed of five phylogenetic groups or complexes: *P. fluorescens*, *P. syringae*, *P. putida*, *P. asplenii*, and *P. lutea* (Garrido-Sanz et al. 2016). Among those, certain species can be beneficial as biocontrol or growth-promoting agents, mostly within the *P. fluorescens* complex, whereas others exhibit phytopathogenicity, mainly belonging to the *P. syringae* complex or the *P. asplenii* group (Höfte and Vos 2007; Weller 2007). The *P. syringae* complex has been divided in more than 60 pathovars and 13 phylogroups, and many members produce diverse phytotoxins, acting as virulence factors in a wide range of hosts (Bender et al. 1999; Xin et al. 2018). These toxins include Lipopeptides (LPs), secondary metabolites composed of a fatty acid tail attached to a peptide (linear, partially, or fully cyclized), predominantly described for phylogroup-2 strains (Berge et al. 2014; Dillon et al. 2019). Typically, two types of cyclic LPs (CLPs), respectively from the "Mycin" and "Peptin" families, and a linear LP (LLP) from the "Factin" family, are produced (Ballio et al. 1988; Ballio et al. 1991; Lindeberg et al. 2008; Burch et al. 2014; Tables 1 and 2). Subsequently, variants belonging to the different LPs families were characterized in diverse phytopathogens, such as *P. cichorii* (*P. syringae* complex, phylogroup-11), *P. fuscovaginae* (*P. asplenii* group), *P. corrugata*, and *P. mediterranea* (*P. fluorescens* complex) (Ballio et al. 1996; Flamand et al. 1996; Emanuele et al. 1998; Scaloni et al. 2004; Licciardello et al. 2012; Pauwelyn et al. 2013; Huang et al. 2015; Götze et al. 2019; Tables 1 and 2). However, CLPs are well known to have anti-microbial properties against a wide range of soil-borne phytopathogens and several "mycin"/

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Supplemental data for this article can be accessed here.

Table 1. *Pseudomonas* LPs from the factin, mycin, and peptin families with known structure.

	Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Factin family																										
Syringafactin A/D	$C_{10/12}$OH	L-Leu	L-Leu	D-Gln	L-Leu	D-*a*Thr	L-Val	D-Leu	L-Leu																	
Syringafactin B/E	$C_{10/12}$OH	L-Leu	L-Leu	D-Gln	L-Leu	D-*a*Thr	L-Leu	D-Leu	L-Leu																	
Syringafactin C/F	$C_{10/12}$OH	L-Leu	L-Leu	D-Gln	L-Leu	D-*a*Thr	L-Ile	D-Leu	L-Leu																	
Cichofactin A/B	$C_{10/12}$OH	L-Leu	L-Leu	D-Gln	L-Leu	D-Gln	L-Val	D-Leu	L-Leu																	
Virginiafactin A/B/C	$C_{10/12}$OH	L-Leu	L-Leu	D-Gln	L-Leu	D-Ser	L-Val/L-Ile	D-Leu	L-Leu																	
Mycin family																										
Syringomycin SRA_1	C_{10}OH	L-Ser	D-Ser	D-Dab	L-Dab	L-Arg	L-Phe	*z*Dhb	L-Asp*	Cl-L-Thr																
Syringomycin E/G	$C_{12/14}$OH	L-Ser	D-Ser	D-Dab	L-Dab	L-Arg	L-Phe	*z*Dhb	L-Asp*	Cl-L-Thr																
Syringostatin A	C_{14}OH	L-Ser	D-Dab	L-Dab	D-Hse	L-Orn	L-*a*Thr	*z*Dhb	L-Asp*	Cl-L-Thr																
Syringotoxin B	C_{14}OH	L-Ser	D-Dab	Gly	D-Hse	L-Orn	L-*a*Thr	*z*Dhb	L-Asp*	Cl-L-Thr																
Pseudomycin A/C	$C_{14/16}$diOH	L-Ser	D-Dab	L-Asp	L-Lys	L-Dab	L-*a*Thr	*z*Dhb	L-Asp*	Cl-L-Thr																
Cormycin A	C_{16}diOH	L-Ser	D-Orn	L-Asn	D-Hse	L-His	L-*a*Thr	*z*Dhb	L-Asp*	Cl-L-Thr																
Nunamycin	C_{14}OH	Ser	Dab	Gly	Hse	Dab	Thr	Thr	Asp*	Cl-Thr																
Thanamycin	C_{16}diOH	Ser	Orn*	Asp	Hse	His	Thr	Dhb	Asp*	Cl-Thr																
Peptin family																										
Fuscopeptin																										
Fuscopeptin A/B	$C_{8/10}$OH	Dhb	D-Pro	L-Leu	D-Ala	D-Ala	D-Ala	D-Ala	D-Val	Gly	D-Ala	D-Val	D-Ala	D-Val	Dhb	D-*a*Thr	L-Ala	L-Dab	D-Dab	L-Phe						
Jessenipeptin	C_{10}OH	Dhb	D-Pro	D-Ile	D-Val	D-Ala	D-Ala	L-Ala	D-Val	D-Ser	D-Ala	D-Val	D-Ala	D-Leu	Dhb	D-*a*Thr	L-Ala	L-Dab	D-Ser	L-Phe						
Corpeptin																										
Corpeptin A/B	$C_{10/12\Delta}$OH	Dhb	Pro	Ala	Ala	Ala	Val	Val	Dhb	Hse	Val	*a*Ile	Dhp	Ala	Ala	Ala	Val	Dhb	*a*Thr	Ala	Dab	Ser	Ile			
Cichopeptin A/B	$C_{12\Delta}$OH	Dhb	Pro	Ala	Ala	Ala	Ala	Val	Dhb	Gly	Val	Ile	Gly	Ala	Val	Ala	Val	Dhb	*a*Thr	Ala	Dab	Ser	Xle Val			
Cichorinotoxin	C_{10}OH	*z*Dhb	D-Pro	D-Ala	D-Ala	D-Ala	D-Val	D-Ala	*z*Dhb	Ala	Val	D-Ile	Ser	Ala	Val	Ala	Val	*z*Dhb	D-*a*Thr	Ala	L-Dab	Ser	Val			
Nunapeptin	C_{12}OH	Dhb	Pro	Ala	Ala	Ala	Val	Ala	Dhb	Ser	Val	Ile	Dhp	Ala	Val	Ala	Val	Dhb	Thr	Ala	Dab	Ser	Ile			
Sclerosin [linear]	C_{10}OH	Dhb	Pro	Ala	Xle	Ala	Val	Val	Dhb	Thr	Val	Xle	Dhp	Ala	Ala	Ala	Val	Dhb	Dhb	Ala	Dab	Ser	Val			
Syringopeptin																										
SP22A/B	$C_{10/12}$OH	*z*Dhb	D-Pro	D-Val	L-Val	D-Ala	D-Ala	D-Val	D-Val	*z*Dhb	D-Ala	D-Val	L-Ala	D-Ala	*z*Dhb	D-*a*Thr	D-Ser	D-Ala	*z*Dhb	L-Ala	L-Dab	D-Dab	L-Tyr			
SP22(SC1/2)	$C_{10/12}$OH	Dhb	Pro	Val	Leu	Ala	Ala	Leu	Val	Dhp	Ala	Val	Ala	Ala	Dhb	*a*Thr	Ser	Ala	Dhb	Ala	Dab	Dab	Tyr			
SP508	$C_{12/14}$OH	Dhb	Pro	Val	Leu	Ala	Ala	Leu	Val	Ala	Ala	Val	Ala	Ala	Dhb	*a*Thr	Ser	Ala	Dhb	Ala	Dab	Dab	Tyr			
SP22(PhvA/B)	$C_{10/12}$OH	Dhb	Pro	Val	Leu	Ala	Ala	Ala	Val	Dhb	Ala	Val	Ala	Ala	Dhb	*a*Thr	Ser	Ala	Dhb	Ala	Dab	Dab	Tyr			
Peptin31	C_{10}OH	Dhb	Pro	Val	Leu	Ala	Ala	–	–	–	–	–	–	–	–	–	–	–	Dhb	Ala	Dab	Dab	Tyr			
Syringopeptin																										
SP25A/B	$C_{10/12}$OH	*z*Dhb	D-Pro	D-Val	L-Ala	D-Ala	L-Val	D-Leu	D-Ala	D-Ala	*z*Dhb	D-Val	*z*Dhb	D-Ala	D-Val	D-Ala	D-Ala	*z*Dhb	D-*a*Thr	D-Ser	D-Ala	L-Val	L-Ala	L-Dab	D-Dab	L-Tyr L-Phe

Shading indicates the AAs responsible for the formation of the ring. The stereochemistry of AAs is indicated when determined. Fatty acid: C_x, x: number of carbon in the chain; Δ: carbon–carbon double bond; OH or diOH: 3-substitutions. Asp*: 3-hydroxy-aspartic acid; Orn*: hydroxylation at the α-carbon of ornithine; Dab: 2,4-diaminobutyric acid; Dha: dehydroalanine; Dhb: 2,3-dehydroaminobutyric acid; Dhp: dehydro-2-aminopropanoic acid; Hse: homoserine; Orn: ornithine; *a*Thr: *allo*-threonine; *a*Ile: *allo*-isoleucine; Xle: Leu or Ile; and Cl-Thr: 4-chlorothreonine. All other AAs are identified by standard three-letter biochemical notation.

Table 2. Original LP profile of representative *Pseudomonas* strains.

Species	Strain	Origin	Accession number	Reference(s)	LP(s)		
					Mycin	Peptin	Factin
***P. syringae* complex**							
P. cichorii	SF1-54	Lettuce (*Lactuca sativa*; Belgium)	KJ513093.1; KJ513094.1	Pauwelyn et al. (2013); Huang et al. (2015)	NA	Cichopeptin	Cichofactin
	JBC1	Soybean (*Glycine max*; South Korea)	CP007039	Yu and Lee (2012); Ramkumar et al. (2015); Götze and Stallforth (2020)	Pseudomycin	Cichopeptin	Cichofactin
	473	Coffee (*Coffea arabica*; Brazil)	QPDS00000000.1	–	Pseudomycin	Cichopeptin-var1	Cichofactin
	MAFF 730229 (YM8705)	Lettuce (*Lactuca sativa*; Japan)	–	Komatsu et al. (2019)	NA	Cichorinotoxin	NA
P. asturiensis	LMG 26898^T	Dark-reddish spots on soybean leaves (*Glycine max*; Spain)	FRDA00000000.1	González et al. (2013)	Absent	Absent	Cichofactin
P. viridiflava	CFBP 1590	Diseased tissue (*Prunus cerasus*; France)	LT855380	Ruinelli et al. (2017)	Absent	Absent	Cichofactin
P. syringae pv. *syringae*	B3A	Peach (*Prunus persica*)	–	Ballio et al. (1991)	Syringomycin	Syringopeptin SP22	NA
	B301D	Flower, pear (*Pyrus communis*)	CP005969	Ballio et al. (1991); Ravindran et al. (2015)	Syringomycin	SP22	Syringafactin
	B728a	Snap bean (*Phaseolus vulgaris*; USA)	CP000075.1	Grgurina et al. (2002); Burch et al. (2014)	Syringomycin	SP22(Phv)	Syringafactin
P. syringae pv. *lachrymans*	508	Apple orchard floor (USA)	–	Grgurina et al. (2005)	NA	SP508 (SP22)	NA
Pseudomonas sp.	p2.C11	*Arabidopsis thaliana* (Germany)	UUIL00000000.1	Karasov et al. (2018)	Syringomycin	SP508 (SP22)	Syringafactin
P. syringae	31R1	Rifampicin-resistant mutant of strain 31	LT629769.1	Lindow (1987); Fiore et al. (2008)	Syringomycin	Peptin31/ SP22-var1*	Syringafactin
P. syringae pv. *syringae*	SC1	Sugar cane (Japan)	–	Fukuchi et al. (1992); Isogai et al. (1995)	Syringomycin	SP-SC1/C2 (SP22)	NA
P. syringae pv. *syringae*	B359 = B497 = HS191	Proso millet (*Panicum miliaceum*; Australia)	CP006256	Ballio et al. (1991); Ballio et al. (1995); Ravindran et al. (2015)	Syringomycin	Syringopeptin SP25	Syringafactin
P. syringae pv. *atrofaciens*	NCPPB 2612 = LMG 5095^T	Wheat (*Triticum aestivum*; New Zealand)	JFZI00000000.1	Vassilev et al. (1997); Bultreys and Gheysen (1999)	Syringomycin	SP25	Syringafactin
P. syringae pv. *syringae*	NCPPB 3869	Laurel (*Laurus nobilis*; Italy)	–	Scaloni et al. (1997)	NA	SP25 (Phe25)	NA
	B427	Lemon (*Citrus*; USA)	–	Ballio et al. (1990); Ballio et al. (1991); Ballio, Collina, et al. (1994)	Syringotoxin	SP25	NA
P. syringae pv. *lapsa*	ATCC 10859	Wheat (*Triticum aestivum*)	CP013183.1	Kong et al. (2016)	Syringotoxin	SP25-var1	Syringafactin
P. syringae pv. *syringae*	SY12	Lilac (*Syringa vulgaris;* Japan)	–	Isogai et al. (1990)	Syringostatin	NA	NA
	3023	Lilac (*Syringa vulgaris*; United Kingdom)	MLFD00000000.1	–	Syringostatin	SP25-var2	Syringafactin
P. syringae	MSU 16H	Elm tree acclimated mutant of MSU 174	–	Lam et al. (1987); Ballio, Bossa, et al. (1994)	Pseudomycin	SP25	NA
P. syringae pv. *syringae*	SM	Wheat (*Triticum aestivum*; USA)	CM001986.1	Smith and Métraux (1991); Dudnik and Dudler (2013)	Pseudomycin	SP25-var3	Syringafactin
P. syringae pv. *tomato*	DC3000	Rifampicin-resistant derivative of DC52, Tomato (*Lycopersicon esculentum*; USA)	AE016853	Berti et al. (2007)	Absent	Absent	Syringafactin
Pseudomonas sp.	SZ57	Soil (Germany)	WIBD00000000.1	Mukherji et al. (2020)	Absent	Absent	Syringafactin
***P. asplenii* group**							
P. fuscovaginae	UPB 264	Rice (*Oryza sativa*; Burundi)	–	Ballio et al. (1996); Flamand et al. (1996)	Syringotoxin	Fuscopeptin	NA

(*continued*)

Table 2. Continued.

Species	Strain	Origin	Accession number	Reference(s)	LP(s)		
					Mycin	Peptin	Factin
P. asplenii	ATCC 23835^{T}	Derived from NRRL B-733; Bird's nest fern (*Asplenium nidus*; USA)	LT629777.1	–	Syringostatin	Fuscopeptin	Absent
P. fuscovaginae	LMG 2158^{T}	Rice (*Oryza sativa;* Japan)	LT629972.1	–	Syringotoxin	Fuscopeptin	Absent
Pseudomonas sp.	QS1027	Fruiting bodies of *Dictyostelium discoideum* (USA)	PHSU00000000.1	Arp et al. (2018); Götze and Stallforth (2020)	Nunamycin-var1	Jessenipeptin	Virginiafactin
P. asplenii	ES_PA-B8	–	RCCF00000000.1	–	Nunamycin-var1	Jessenipeptin	Virginiafactin
***P. mandelii* group**							
P. fluorescens	In5	Soil of potato (*Solanum tuberosum*; Greenland)	LIRD00000000.1	Michelsen, Jensen et al. (2015); Michelsen,Watrous, et al. (2015)	Nunamycin	Nunapeptin	Absent
***P. corrugata* group**							
P. corrugata	IPVCT 10.3	Tomato (*Lycopersicon esculentum*; Italy)	–	Scaloni et al. (2004)	Cormycin	NA	NA
	LMG 2172 = NCPPB 2445^{T}	Tomato (*Lycopersicon esculentum*; UK)	RBOJ00000000.1	Emanuele et al. (1998)	Thanamycin	Corpeptin	Absent
P. fluorescens	DSM 11579	Soil (USA)	KT362216.1	Johnston et al. (2015)	Thanamycin	NA	NA
Pseudomonas sp.	SH-C52	Sugar beet (*Beta vulgaris*; The Netherlands)	CBLV000000000.1	Van Der Voort et al. (2015)	Thanamycin	Thanapeptin	Absent
P. fluorescens	7SR1	Sugar beet (*Beta vulgaris*; USA)	LT707064.1	Melnyk et al. (2019)	Thanamycin	Thanapeptin	Absent
P. brassicacearum	11K1	Rhizosphere of *Vicia faba* (China)	CP035088.1	Zhao et al. (2019)	Brasmycin	Braspeptin	Absent
P. kilonensis	ZKA7	Greece	QEKL00000000.1	–	Thanamycin	Braspeptin	Absent
P. brassicacearum	DF41	Root of canola (*Brassica napus*; Canada)	CP007410.1	Berry et al. (2012); Berry et al. (2014)	Thanamycin-var1	Sclerosin	Absent
P. mediterranea	CFBP 5447 = DSM 16733^{T}	Tomato (*Lycopersicon esculentum*; Italy)	LT629790.1	–	Thanamycin	Peptin 22-var1	Absent
Pseudomonas sp.	FW300-N2C3	Ground water (USA)	CP012831.1	Melnyk et al. (2019)	Thanamycin	Peptin 22-var2	Absent
	Pf153	Tobacco roots (*Nicotiana tabacum*; Switzerland)	LHVL00000000.1	Fuchs (2000)	Thanamycin	Peptin-13	Absent

Chemically characterized LPs are indicated by shading and other LPs are based on predictions (Tables S1–S3). LP-8 corresponds to the predicted LP brasamide described by Zhao et al. (2019). Dashes indicate strains for which the corresponding information is not available. In *P. syringae* 31R1, SP22-var1 is highlighted by an asterisk to indicate that, in contrary to peptin31, it has not yet been chemically characterized. NA: genomic sequence not available (presence or absence of additional LP BGCs unknown).

"peptin" producers within the *P. fluorescens* complex (*P. corrugata* and *P. mandelii* groups) have been proposed as biocontrol agents (Berry et al. 2014; Michelsen, Jensen, et al. 2015; Van Der Voort et al. 2015). In fact, the distinction between beneficial and phytopathogenic LP producers is not very strict and, even if CLPs have been described as virulence factors among well-known pathogens, the role of these molecules in other *Pseudomonas* species remains unclear.

LPs are assembled by non-ribosomal peptide synthetases (NRPSs) encoded by large biosynthetic gene clusters (BGCs) and many *Pseudomonas* strains possess multiple BGCs coding for the production of mycins, peptins, and/or factins (Gross and Loper 2009). However, for a particular producer, LPs are often chemically characterized one by one, with or without the stereochemistry, the corresponding BGCs and the biological activities, which makes the knowledge very disparate and the comparison arduous (Ballio et al. 1988; Ballio et al. 1991; Ballio et al. 1996; Emanuele et al. 1998). Intensive efforts were recently made in order to gather such information for a large number of LP families but the mycin/peptin/factin producers were not included or the diversity of their BGCs was not examined in detail (Geudens and Martins 2018; Götze and Stallforth 2020). In this review, we make the link between LP structures, BGC organizations and diversity, and biological activities for these families. This work has led us to complement the current knowledge with a non-exhaustive genome mining-based inventory to offer a comprehensive genus-wide overview on these LPs producers. Moreover, it allowed us to present a general comparison of BGC evolutionary relationships and to highlight gaps necessary to fill in order to clarify the boundary between beneficial and detrimental plant-*Pseudomonas* relationships.

2. Phytopathogenic *Pseudomonas*: LPs as virulence factors

2.1. Two different biosynthetic strategies for LP production in P. syringae

Several *P. syringae* strains co-produce LPs from the mycin, peptin, and factin families. In the late 1980s and early 1990s, the chlorinated CLPs syringomycin, syringostatin, syringotoxin, and pseudomycin were characterized (Ballio et al. 1988; Ballio et al. 1990; Ballio, Bossa, et al. 1994; Ballio, Collina, et al. 1994; Segre et al. 1989; Scaloni et al. 1994). These members of the mycin family are composed of a fatty acid tail ranging from 10 to 16 carbons attached to a fully cyclized peptide, made of nine amino acids (AAs), with the last being a chlorinated threonine (Table 1). The peptin family encompasses the largest known CLPs, and in *P. syringae* strains these compounds are made of a partially cyclized peptide (8-ringed) with either 22 AAs in syringopeptin SP22 (Ballio et al. 1991) and its variants SP22(SC) (Isogai et al. 1995), SP508 (Grgurina et al. 2005), and SP22(Phv) (Grgurina et al. 2002), or 25 AAs in syringopeptin SP25 (Ballio et al. 1991; Ballio et al. 1995) and it is variant SP25(Phe) (Scaloni et al. 1997). The factin family comprises LLPs with eight AAs and its first member, syringafactin, was identified in diverse *P. syringae* strains (Berti et al. 2007; Burch et al. 2014; Tables 1, 2, and S1).

The LPs of the peptin and factin families are synthesized co-linearly with the order of the modules present in their respective NRPS enzymes. The same biosynthesis scheme is used for the production of most *Pseudomonas* CLPs (reviewed by Götze and Stallforth 2020). A typical module is composed of three domains ordered as [C-A-T]: C (AA condensation), A (AA-specific adenylation), and T (AA thiolation for thioester binding). The fatty acid is attached to the first AA by a dedicated condensation domain with *N*-acylation activity (C1 or $C_{starter}$ mediating lipoinitiation). Subsequent additions of AAs are catalysed by either a regular condensation domain ($^{L}C_{L}$ domain connecting two L-configured AAs) or, more frequently, by a condensation domain with built-in epimerization activity (C/E domain). The latter converts the configuration of the previously incorporated residue from L to D, avoiding the need for separate epimerization (E) domains, such as those present in most *Pseudomonas* pyoverdine synthetases (Visca et al. 2007). Release from the terminating enzyme, with concomitant cyclization in the case of CLPs, is catalysed by a thioesterase (TE) domain. Like most CLP systems in *Pseudomonas*, the peptin and factin terminal NRPSs carry a tandem of TE domains, while mycin synthetases only possess one TE domain (Figure 1(A, C, and D)). Elucidation of the syringomycin biosynthetic pathway has revealed a number of distinguishing features (Götze and Stallforth 2020; Jaremko et al. 2020). Syringomycin synthetase SyrE lacks an A-domain in the last module [C-T] which is provided by a separate enzyme with an [A-T] module (SyrB1; Figure 1(A,B); Myc-a-1). Threonine attached to SyrB1 is chlorinated by SyrB2 and then shuttled *via* SyrC to the last T-domain of SyrE for condensation of the co-linear octapeptide intermediate with L-4-chlorothreonine prior to cyclization. Yet another stand-alone enzyme (SyrP) participates in biosynthesis by hydroxylation of aspartate, serving as a substrate for the penultimate module of SyrE.

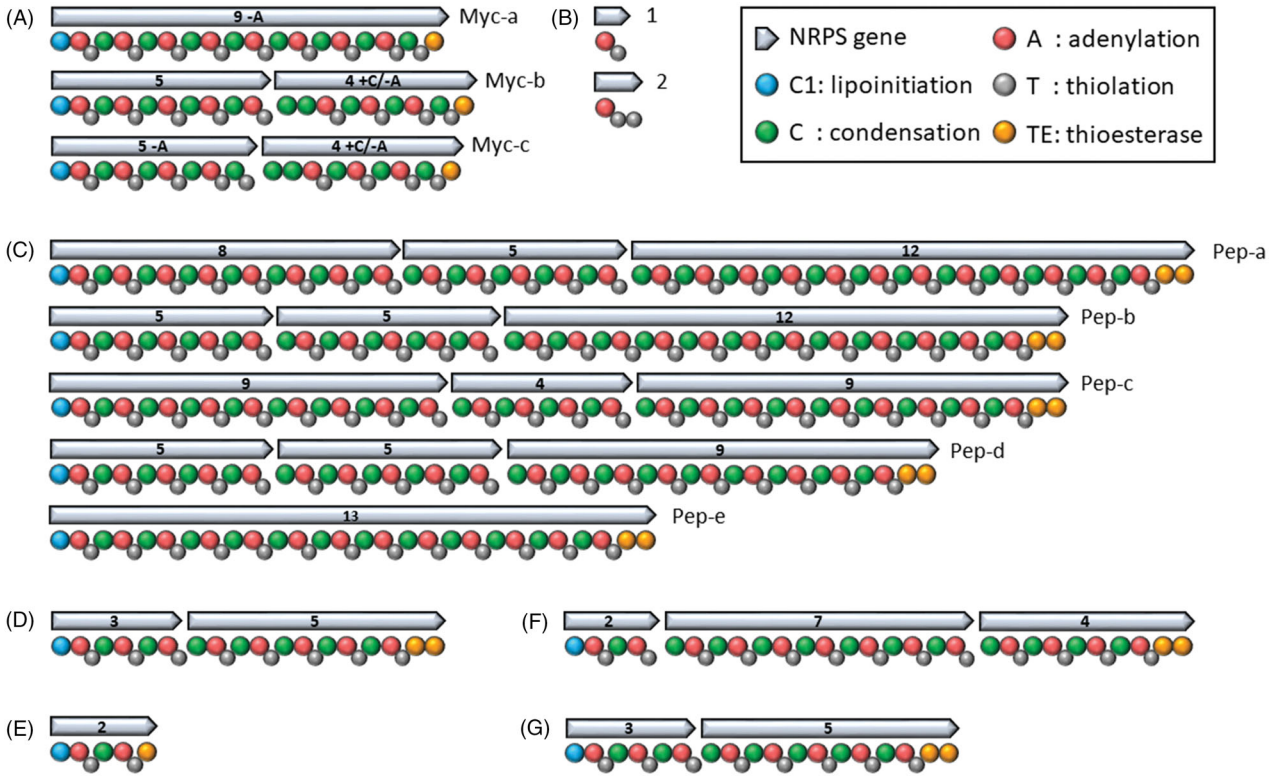

Figure 1. Module architecture of *Pseudomonas* NRPS enzymes involved in the biosynthesis of mycins (A and B), peptins (C), factins (D), brabantamide (E) and predicted LP-13 (F), and LP-8 (G). Representative domain configurations are shown, differing by the presence of a single (A and E) or tandem TE-domain (C, D, F, and G) and the number of regular [C-A-T] modules encoded by the respective genes (specified inside arrows). Deviations from the canonical module composition in mycin systems are highlighted: presence of an additional C-domain (+C); absence of an A-domain (−A). A variant of the regular SyrB1 module [A-T] (B:1) is expanded with an extra T-domain (B:2). The different enzyme configurations occurring in the mycin and peptin family are specified for the respective CLPs in Tables S2 and S3.

2.2. P. syringae's CLP island

In the genomes of the syringomycin producers *P. syringae* pv. *syringae* B301D (co-producing SP22) and B728a (co-producing SP22(Phv)), both BGCs are tightly clustered (Figure 2). Similarly clustered BGCs are also present in the genomes of SP25-producing strains such as *P. syringae* pv. *syringae* HS191 and *P. syringae* pv. *atrofaciens* LMG 5095^T. The SP22 and SP25 NRPS systems display similar organization with genes *sypB* (5 modules) and *sypC* (12 modules) but differ in *sypA* (five *versus* eight modules). SP25-type NRPS genes are also present in *P. syringae* pv. *lapsa* ATCC 10859, but the adjacent Mycin cluster appears to have a split equivalent, SyrE1 (five modules) and SyrE2 (four modules) (Myc-b-1, Figures 1(A,B) and 2), of the common syringomycin synthetase SyrE (nine modules)(Myc-a-1, Figure 1(A,B)). In *P. syringae* pv. *syringae* SM, compared to other SP25-type BGCs (HS191and LMG 5095^T), the *syrE* gene is lost. Apparently, this loss is compensated by the acquisition of a different type of Mycin cluster (*syrE1syrE2*; similar to ATCC 10859; Myc-b-1, Figure 1(A,B)). The latter NRPS genes are located about 300 kb upstream of the original *syrE* and on the opposite strand (Figure 3). All these phylogroup-2 *P. syringae* strains carry a syringafactin BGC at about 34–38 kb downstream of their syringomycin NRPS gene(s). However, other *P. syringae* strains only harbour a factin BGC (lacking the mycin and peptin BGCs) such as the tomato pathogen *P. syringae* pv. *tomato* DC3000 (phylogroup-1) or *Pseudomonas* sp. SZ57 (phylogroup-2) (Berti et al. 2007; Mukherji et al. 2020).

In addition, a cryptic BGC (Hybrid NRPS-PKS-1, Figure 2) comprising two NRPS genes and a hybrid NRPS-PKS gene can be present. Its second and third enzyme are homologous to RmoH and RmoI (at 44 and 48% AA identity, respectively) encoded by a syntenic gene pair required for synthesis of rimosamide by *Streptomycetes* (McClure et al. 2016).

2.3. LP archipelago of P. cichorii

Distinct members of the peptin and mycin families have been identified in the lipopeptidome of *P. cichorii*,

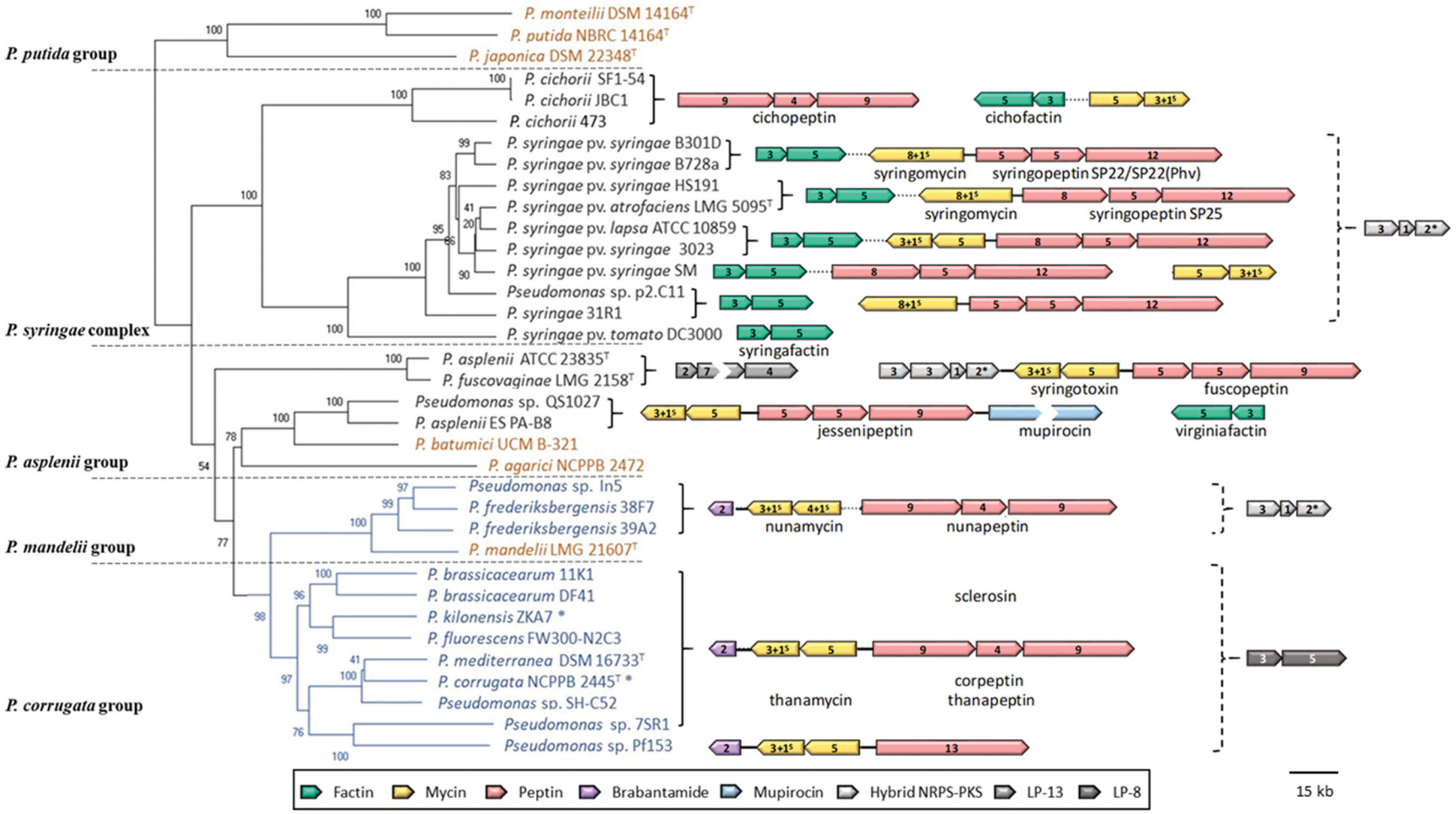

Figure 2. Overview of the NRPS-based systems for synthesis of LPs of the mycin, peptin, and factin families in *Pseudomonas*. The phylogenetic tree is based on the concatenated analysis of 16S rRNA, *gyrB*, *rpoB*, and *rpoD* genes (Mulet et al. 2010). The distance matrix was calculated by the Jukes-Cantor method and the dendrogram was generated by neighbour joining (NJ, bootstrap 1000). Phylogenetic groups/complexes are delineated (dashed lines). Three strains of the *P. putida* group were included as an outgroup. Reference strains are indicated in orange and correspond to those used by Hesse et al. (2018). Strains harbouring the LPQ island organization are highlighted in blue and an asterisk indicates those without the additional LP-8 BGC. The LPQ genomic sequence of thanamycin producer *P. fluorescens* DSM 11579 (not shown) is nearly identical (99%) to the one of *Pseudomonas* sp. 7SR1. The arrows represent NRPS genes and are coloured according to the type of CLP synthesized. The number of modules for incorporation of consecutive amino acids by the respective encoded NRPS enzymes is indicated. The number "1^{S}" refers to a module lacking an A-domain (see Figure 1). Chemically characterized LPs are indicated in the figure (names aligned horizontally and vertically with corresponding producer and BGC, respectively) and listed, together with the peptide sequence of predicted ones, in Table 1. The mupirocin PKS BGC is drawn without individual genes and not to scale (broken arrow). Two types of uncharacterized hybrid NRPS-PKS systems, Hybrid NRPS-PKS-1 (three genes) and Hybrid NRPS-PKS-2 (four genes), are shown in light grey and their genes encoding a NRPS with carboxyterminal PKS domains are marked with an asterisk. Solid connectors between genes correspond to a distance <10 kb, dotted connectors 10–50 kb and blank space >50 kb. A detailed version of the BGCs, where described NRPS genes are labelled, is available in Figure S1.

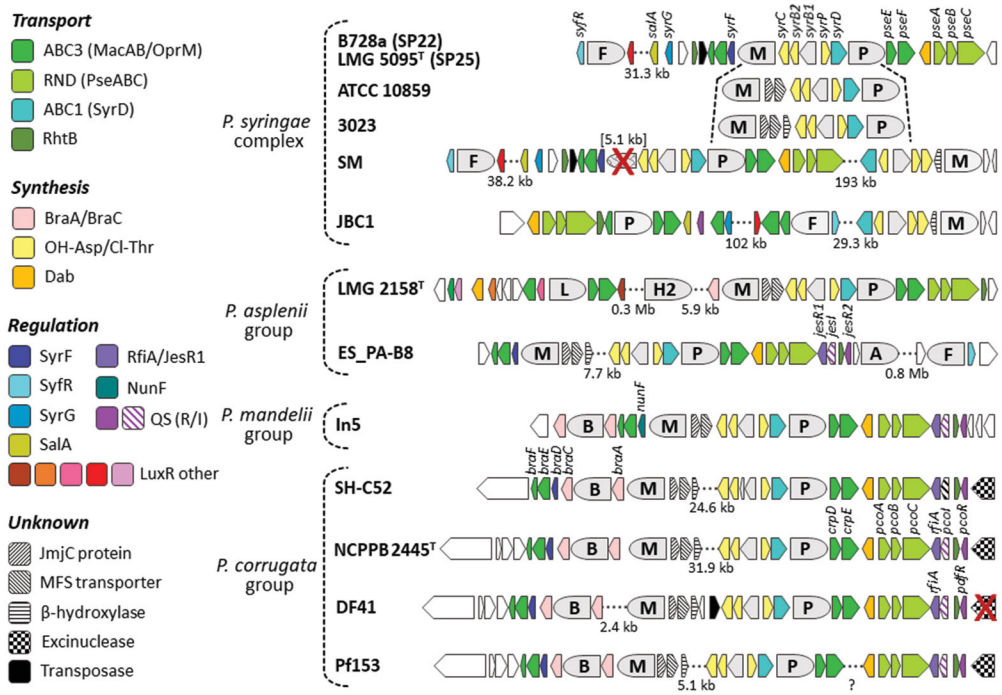

Figure 3. Genetic organization of the regions flanking representative *Pseudomonas* BGCs for LPs of the mycin, peptin, and factin families. The grey bullets represent the NRPS enzymes specified in Figure 2 (not to scale): B, brabantamide; F, factin; L, LP-13, M, mycin; P, peptin; A similar representation is used for the Hybrid NRPS-PKS-2 system (H2) and the mupirocin PKS system (A). The distances between non-adjacent genes (separated by a dotted line) are specified. The colour legend specifies the respective gene products, with the same colour used for homologs present in different strains. Genes that are discussed in the text are labelled. Pseudogenes or gene remnants are crossed. The distance between the thanamycin and thanapeptin clusters differs between strains SH-C52 (24.6 kb) and DSM 11579 (2.8 kb, not shown). The BGC organization of *P. syringae* strains ATCC 10859 and 3023 is identical to those of strains B728a and LMG 5095^T, except for two or three additional genes in the region between the M and P clusters (only the latter part is shown).

another phytopathogen belonging to *P. syringae* complex. *P. cichorii* SF1-54 produces cichopeptin, also made of 22 AAs but differing from SP22 by the size of its macrocycle (five *versus* eight; Table 1) and its AA composition (Huang et al. 2015), and cichofactin which differs from syringafactin by a single AA (Table 1, Pauwelyn et al. 2013; Götze et al. 2019). Molecular mass data suggest that a third mycin BGC yields pseudomycin (Ballio, Bossa, et al. 1994; Pauwelyn 2012). The genome of the closely related cichofactin producer *P. cichorii* JBC1 reveals that the tightly organized mycin-peptin BGCs, typical for most *P. syringae* strains, are not observed here (Götze et al. 2019). The three BGCs occur separately in the genome, hence the suggestion of an "archipelago" (Figure 2). Interestingly, the Mycin BGC shows striking synteny with the one of *P. syringae* pv. *syringae* SM. The cichopeptin analogue produced by *P. cichorii* MAFF 730229 diverges at four different position and it will be of interest to genome sequence this strain for comparative analysis (Komatsu et al. 2019; Table 1).

2.4. *P. fuscovaginae*: standing out from the phytopathogenic crowd

A more distant relative of *P. syringae* that produces CLPs from the Mycin and Peptin families is the rice pathogen *P. fuscovaginae*. Fuscopeptin produced by *P. fuscovaginae* UPB 264 resembles cichopeptin by its cyclization pattern (5-ringed) but differs by its shorter and different peptide sequence of 19 AAs (Ballio et al. 1996; Table 1). This strain produces syringotoxin (Flamand et al. 1996; Table 1), which is also reported in some *P. syringae* strains (Ballio et al. 1990; Fukuchi et al. 1992; Table 2). In *P. fuscovaginae* LMG 2158^T, the corresponding BGCs occur in a single cluster. This strain lacks a BGC for factin production but also carries a cryptic rimosamide-related BGC, however, equipped with an extra triple-modular NRPS gene (Hybrid NRPS-PKS-2, Figure 2). Another cryptic BGC consists of a tripartite NRPS operon that would generate a novel lipotridecapeptidic product (LP-13) (Figures 1(F) and 2, Table S1). The structure and function of this molecule will be pub-

lished elsewhere. Nearly identical syntenic BGCs are present in the genome of *P. asplenii* ATCC 23835^T, suggesting that these closely related species, have a very similar capacity to produce several (lipo)peptidic secondary metabolites (Hu et al. 1998). It should be noted that Tohya et al. (2020) recently suggested that *P. fuscovaginae* should be classified as a later heterotypic synonym of *P. asplenii* since the average nucleotide identity between the two type strains representing these species is 98.4%.

A fuscopeptin-related compound, jessenipeptin, was recovered from *Pseudomonas* sp. QS1027, another member of the *P. asplenii* group, isolated from *Dictyostelium discoideum* fruiting bodies (Arp et al. 2018; Table 1). A distinguishing feature of this peptin producer is the presence, immediately downstream of the jessenipeptin cluster, of a large BGC (73.7 kb with 34 ORFs) for the known polyketide antibiotic mupirocin. To reconstruct the probable organization of the fragmented jessenipeptin BGC and its missing upstream region, we mapped several contigs of the QS1027 draft genome on a large contiguous genomic sequence of the closely related strain *P. asplenii* ES_PA-B8, also carrying near-identical mupirocin and jessenipeptin BGCs (Figure S2). This assembly also suggests that a Mycin BGC is located upstream of the jessenipeptin cluster (Myc-b-2, Figures 1(A,B) and 2). The unlinked factin-type BGC of QS1027 yields virginiafactin (Götze et al. 2019; Table 1). Neither strain appears to accommodate the rimosamide-related BGC of the *P. asplenii* and *P. fuscovaginae* type strains.

Interestingly, the MLSA phylogeny reveals a split *P. asplenii* group with *P. asplenii* ATCC 23835^T and *P. fuscovaginae* LMG 2158^T being separated from *P. asplenii* ES_PA-B8 and *Pseudomonas* sp. QS1027, in line with changes in BGCs composition and organization that have occurred during their divergent evolutionary history (Figure 2). Strain ES_PA-B8 has only 84% average nucleotide identity with *P. asplenii* ATCC 23835^T and needs to be reclassified (Tohya et al. 2020).

3. *P. fluorescens* complex

3.1. LPQ: an island not merely linked to phytopathogenicity

Within the *P. fluorescens* complex, CLPs of the Peptin family were characterized in strains affiliated with the *P. corrugata* and *P. mandelii* groups, e.g. corpeptin (Emanuele et al. 1998), nunapeptin (Michelsen, Watrous, et al. 2015), thanapeptin (Van Der Voort et al. 2015), and the LLP sclerosin (Berry et al. 2012; Tables 1 and 2). Their structures, all containing a 22-AA peptide, bear more similarity to cichopeptin than to the shorter fuscopeptin/jessenipeptin and this is reflected in similar NRPS organizations: 9 – 4 – 9 modules for cichopeptin and cor/nuna/thanapeptin *versus* 5 – 5 – 9 for fusco/jessenipeptin (Figure 2). On the other hand, *Pseudomonas* sp. Pf153 harbours a single NRPS gene coding for 13 modules for the synthesis of a putative short, possibly linear peptin (peptin-13 in Table 1; Fuchs 2000).

Consistently, these strains co-produce a second CLP from the Mycin family, e.g. cormycin (Scaloni et al. 2004), nunamycin (Michelsen, Watrous, et al. 2015), or thanamycin (Watrous et al. 2012; Johnston et al. 2015) (Table 1). The corresponding mycin clusters encode two NRPSs whose module architecture can be differentiated by the phylogenetic affiliation of the producer (Figures 1(A,B) and 2), e.g. *P. corrugata* group (Myc-b-1) and *P. mandelii* group (Myc-c-1). Their respective Mycin and Peptin BGCs are tightly packed in *P. fluorescens* In5 and *P. brassicacearum* DF41, or with some non-conserved intervening region, ranging from 2.8 kb in thanamycin producer *P. fluorescens* DSM 11579 to about 32 kb in *P. corrugata* NCPPB 2445^T (Figure 3). In addition, members of the *P. mandelii* group, similarly to *P. syringae* strains, possess an unlinked rimosamide-related BGC (Hybrid NRPS-PKS-1) and most of the strains belonging to the *P. corrugata* group harbour a cryptic BGC consisting of a bipartite NRPS operon that would generate a novel lipo-octapeptidic product (LP-8, Figures 1(G) and 2, Table S1).

The closely co-integrated tandem BGCs organization is also present in *Pseudomonas* sp. FW300-N2C3. Although the corresponding metabolites have not yet been characterized, Melnyk et al. (2019) showed that this genomic region is required for phytopathogenic effects in a gnotobiotic assay, also dependent on the activity of the associated *quorum sensing* (QS) genes. Hence, it was considered to represent a pathogenicity island, designated LPQ (lipopeptide/QS) island, conserved among members of the *P. corrugata* and *P. mandelii* groups (Table 2 and Figure 2). Interestingly, it was suggested that the gain or loss of the LPQ island might occur by homologous recombination. A putative exci-nuclease gene (*uvrA2* homolog) is flanking the peptin BGC in the *P. corrugata*-type LPQ, but it is not known whether this may be functionally linked to the mobility of this island (Tark et al. 2008). Additionally to the peptin/mycin BGC, the LPQ island harbours QS genes (*luxR*/*luxI*) downstream of the peptin cluster and a brabantamide BGC downstream of the Mycin cluster (Figures 2 and 3).

3.2. Brabantamide: an immigrant BGC settled on the LPQ island

The *braABC* operon is conserved among strains harbouring a LPQ island organization and was previously considered to be part of the "syringomycin synthetase operon" (Melnyk et al. 2019; Figure 3). This BGC was independently shown to drive the synthesis of the cyclocarbamate antibiotic brabantamide in *Pseudomonas* sp. SH-C52 (Schmidt et al. 2014) and *P. fluorescens* DSM 11579 (equivalent operon *lpiABC*; Johnston et al. 2013). The lipodipeptide core of the molecule is assembled by a dual-module NRPS (BraB, Figure 1(E)) and further modified by the monooxygenase BraC and the rhamnosyl transferase BraA. Two LPQ variants have evolved in the *P. corrugata* and *P. mandelii* groups by integrating the brabantamide BGC at a slightly shifted position, respectively, in front or behind a conserved triplet of genes involved in regulation and transport (see Sections 5 and 6). Compared to the SH-C52 enzymes, the BraBC proteins in *P. corrugata* and *P. mandelii* groups show strong sequence conservation (>95 and >80% identity, respectively).

Within the *P. asplenii* group, *P. fuscovaginae* LMG 2158^T and *Pseudomonas* sp. QS1027, harbour differently organized brabantamide-like genes. Indeed, in *P. fuscovaginae* LMG 2158^T only *braA* is found downstream the mycin cluster (Figure 3), and two sets of *braBC* homologous are present: one upstream the LP-13 coding for a bi-modular *braB* and one far downstream the mycin-peptin BGC coding for a tri-modular *braB*. In *Pseudomonas* sp. QS1027 no *braA* was retrieved but a *braBC* set coding for a bi-modular BraB is present. The divergent NRPS sequences present in these strains (<45% identity to SH-C52 BraB) suggest that the structure of the corresponding compounds is likely different from brabantamide and needs further characterization.

4. Prominent differences between mycin and peptin/factin families

4.1. BGC organization and NRPS architecture

The peptin and factin NRPS systems are quite similar to those of most other CLP families in *Pseudomonas*, only differing by the overall number of modules and their distribution across one to three NRPS gene(s) (Figures 1 and 2; Götze and Stallforth 2020). A very similar modular design is apparent for the unlinked NRPS systems predicted to assemble a lipotridecapeptide (LP-13) or a lipo-octapeptide (LP-8).

The well-characterized syringomycin NRPS system is atypical for most *Pseudomonas* CLPs as it comprises one large mega-enzyme (SyrE) complemented with an external [A-T] enzyme (SyrB1) (Myc-a-1; Figure 1(A,B)). However, in several known and predicted mycin producers architectural variations are noted. In most strains, including some *P. syringae* SP25 producers, the mycin BGC encodes two synthetases, further denoted as SyrE1 and SyrE2 (Figure 2). Such SyrE2 retains the "split" architecture of the last module but features a tandem C-domain [C-C-A-T] in the first module (Myc-b; Figure 1(A)). A further deviation from the syringomycin model, the lack of an A-domain in the last module of the first synthetase (Myc-c; Figure 1(A)), was first noted for nunamycin and module shuffling (a module functioning twice) was put forward as a possible explanation (Michelsen, Watrous, et al. 2015). This domain architecture is also present in *P. cichorii* 473 but not in other inspected *P. cichorii* strains. For representative strains, the Myc subtypes and occurrence of an atypical [A-T-T] SyrB1 (Figure 1(B:2)) are documented in Table S2.

The conserved module composition of SyrE and concatenated SyrE1-E2 enables a global comparison of mycin NRPSs by phylogenetic analysis (Figure 4). Among strains belonging to the *P. syringae* complex the mycin synthetases are distributed across distant clades and branches. The SyrE1-SyrE2 pair of *P. syringae* pv. *syringae* SM clusters with the *P. cichorii* enzymes (about 80% AA identity), in line with the striking synteny of their uncoupled mycin BGCs predicted to generate pseudomycin. The SyrE enzymes mediating syringomycin biosynthesis constitute a well-resolved clade, separated from two branches with concatenated SyrE1-SyrE2 enzymes from the potential producers of syringostatin, and syringotoxin. With the latter *P. syringae* enzymes, the syringomycin synthetases share only about 60% AA identity and similarity is even lower (48% AA identity) to strain SM pseudomycin synthetases. The SyrE1-SyrE2 sequences from the LPQ strains separate into two distinct clades (on average 66% AA identity) that reflect their differentiated CLP synthesis (nunamycin or thanamycin/thanamycin variant) and phylogenetic affiliation (*P. mandelii* or *P. corrugata* group). Similarly to the MLSA phylogeny of the *P. asplenii* group (Figure 2), the NRPS concatenate phylogeny reveals two branches with (i) strains QS2017 and ES_PA-B8 potentially producing a new nunamycin variant and (ii) strains ATCC 23835^T and LMG 2158^T, respectively, predicted to produce syringostatin and syringotoxin. Interestingly, the enzymes of the last two strains are predicted to assemble the same peptide moiety as those of the *P. syringae* strains 3023 and

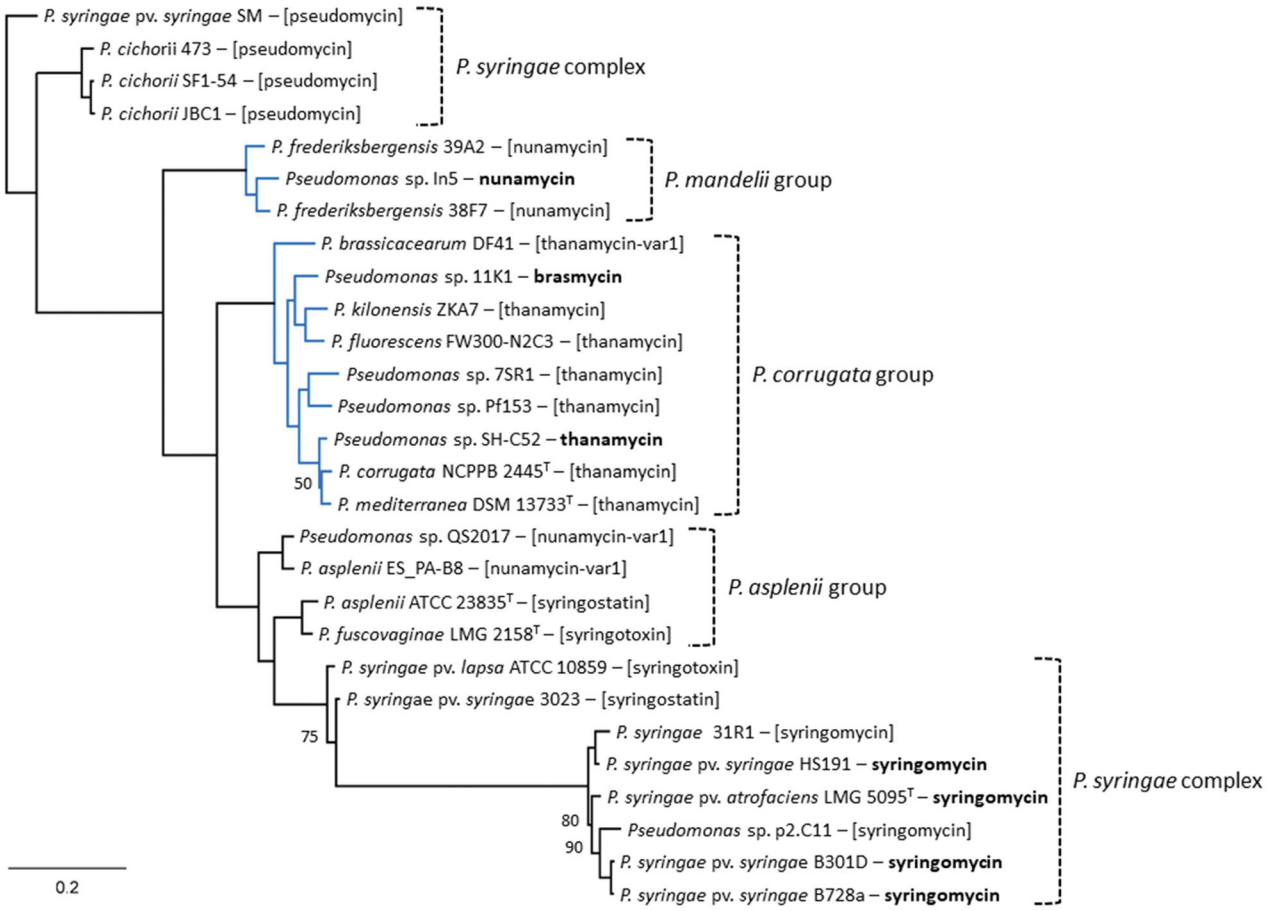

Figure 4. Phylogenetic analysis of mycin synthetases. Maximum-likelihood phylogenetic tree (PhyML, JTT substitution model) inferred from multiple AA sequence alignment of single SyrE and concatenated SyrE1-SyrE2 homologous of representative *Pseudomonas* strains belonging to different phylogenetic groups/complexes (delineated by labelled right brackets; see Figure 2). The separate SyrB1 sequences are not included in the comparison. The LPQ cluster enzymes are indicated in blue. The DSM 11579 enzymes (not shown) share 99.7% AA identity with the 7SR1 orthologous. Characterized CLPs produced by these NRPS systems are shown in bold next to the corresponding producer strain. Other CLPs tentatively assigned based on the same or a similar predicted peptide sequence are shown in double brackets. The predicted AA sequence of *P. corrugata* NCPPB 2445^T matches the one of thanamycin (Asp-3), whereas cormycin of original producer IPVCT 10.3 incorporates Asn-3. Only bootstrap values (percentages of 100 replicates) below 100 are shown. The scale bar represents 0.2 substitutions per site.

ATCC 10859, but their biosynthetic systems show host group-associated divergence (about 84% AA identity).

4.2. Peptide stereochemistry

The overall peptide stereochemistry in CLPs from the mycin/factin families shows a higher proportion of L-configured residues, setting them apart from those of the peptin family (and most other *Pseudomonas* CLPs) with predominantly D-AAs. In the mycin family, this is reflected in the balance between $^{L}C_{L}$ and C/E domains in their NRPS enzymes (Tables S2 and S3). It should be noted that phylogeny-based classification of C-domains for stereo-chemical prediction has its limitations, as some of the experimentally determined AA-configurations conflict with the predicted ones (Balibar et al. 2005, "Balibar exceptions", Tables S2 and S3). The starter C1-domain attaches the first AA to the fatty acid, most frequently a 3-hydroxy derivative but chain length can range from C8 to C16. Dihydroxy and mono-unsaturated fatty acids are less frequent (Table 1). A similar starter C1-domain is active in brabantamide biosynthesis. A C1-domain cladogram shows that sequences group consistently according to family affiliation of the NRPS and not according to chemical nature of the selected fatty acid moiety (Figure S3).

4.3. Conserved accessory and unknown genes

The non-proteinogenic residue Dab (2,4-diaminobutyric acid) is incorporated in all characterized peptins and most mycins (Tables S2 and S3) and the *dab* gene is present in the BGCs of all known (and predicted) peptin-mycin co-producers (Walsh et al. 2013; Figure 3). In

P. syringae pv. *syringae* B301D, the *dab* gene is co-transcribed with biosynthesis and transport genes and is required for CLP production (Lu et al. 2002). In *P. fuscovaginae* it appears uncoupled from the fuscopeptin genes and integrated in the regulatory region of the cryptic LP-13 BGC (Figure 3). It has not yet been elucidated which enzymes generate the three other non-proteinogenic AAs, Dhb (2,3-dehydroaminobutyric acid), Dhp (dehydro-2-aminopropanoic acid), and Dha (dehydroalanine) (Grgurina and Mariotti 1999; Götze and Stallforth 2020).

Synteny analysis of BGCs for mycins and peptins production revealed a set of 2 or 3 convergent genes of unknown function that are found, in many producers, right upstream of the mycin cluster. The two first genes encode for, respectively, a putative membrane-anchored protein with a periplasmic JmjC-family hydroxylase domain (Gao et al. 2018) and an unknown MFS efflux protein (DHA3 family; transporter classification TC 2.A.1.21). The third gene, absent from some clusters (e.g. nunamycin) or present individually (*P. syringae* SM and *P. cichorii* strains), is encoding a member of the β-hydroxylase family (Pfam PF05118) for which no function has yet been assigned (Reitz et al. 2019; Figure 3).

5. Multiple complementary systems for CLP export

Multiple transport-related genes are integrated in the mycin/peptin BGCs and they are co-expressed with the biosynthetic genes (Lu et al. 2005; Licciardello et al. 2018). The first is a tripartite export system required for CLP secretion, made of a cytoplasmic membrane protein (MacB), a periplasmic adaptor (MacA), and an outer-membrane protein (OMP) (Lu et al. 2005; Strano et al. 2015; Greene et al. 2018; Licciardello et al. 2018). The *macAB* gene pair (also *pseEF* or *crpDE*) is consistently situated downstream of the third Peptin gene (*sypC* and *crpC*) and the gene likely encoding the required OMP (*nunGX* in the *P. mandelii* group) is located downstream of the Mycin synthetase (*syrE* gene(s); Figure 3). The embracement of the NRPS BGCs by transporter genes is reminiscent of the typical gene organization in *Pseudomonas* strains, irrespective of the CLP family. Such "transporter hug", consisting of well-conserved transporter genes flanking a BGC or BGC cluster at the distal NRPS genes, is even maintained if the NRPS genes are present at two distant genomic locations (Figure 3). Similarly, within the *P. corrugata* group, the OMP genes are located downstream of the brabantamide operon (*braEF*), thus including the brabantamide BGC into this transporter hug. On the other hand, when the factin cluster is the only LP BGC, a *macAB* pair (*syfCD*) is present right downstream the biosynthetic genes (*syfAB*) and mediate the transport (Berti et al. 2007). This may explain why a second *macAB* module is still part of the separate cichofactin and LP-13 BGCs (respectively, in *P. cichorii* and *P. fuscovaginae*), whereas in others (e.g. *P. syringae* and *P. asplenii*) the export system seems to have evolved towards dual use, accommodating the export of factins and peptins.

A second secretory route is specified by homologs of the *P. syringae* genes *pseABC*, located in the downstream region of the *macAB* genes only separated by the *dab* gene (Figure 3). This second tripartite export system, made of a pmf-dependent RND-superfamily transporter (PseC; TC 2.A.6.3.8), a periplasmic adaptor (PseB), and an OMP (PseA), appears necessary for syringomycin and syringopeptin secretion (to a greater extent for syringopeptin; Kang and Gross 2005), and the corresponding genes are co-transcribed with the respective biosynthetic genes (Lu et al. 2005; Licciardello et al. 2018). However, the similarities between the different components of these two first efflux systems are quite low (<25–30% identical AAs).

A third type of transporter protein, SyrD, also designated cyclic peptide transporter, encoded by the *syrD* gene, located in the conserved intergenic region between the syringomycin and syringopeptin BGCs, was shown to be required for syringomycin production (Quigley et al. 1993; Figure 3). SyrD is similar to the ABC transporter PvdE, involved in the export of the pyoverdine precursor pseudobactin to the periplasm (Cornelis 2010). By analogy with PvdE, SyrD might serve to support the transport of (a precursor of) syringomycin to the periplasm, where it may be further translocated *via* the RND transport system PseABC. In strains with less tightly organized mycin/peptin BGCs, the *syrD* gene is nevertheless located in the 5′-region of the peptin operon. *P. cichorii* again shows a deviation from this common organization with the *syrPD-syrB1B2C* cluster being associated with the unlinked mycin BGC. Remarkably, in *P. syringae* pv. *syringae* SM a syntenic mycin BGC is present, apparently acquired to replace the original "regular" *syrE* that has been largely deleted without the loss of the associated *syrPD-syrB1B2C* (Figure 3). As a result, this strain accommodates two clusters, an "indigenous" one associated with the peptin BGC and an "exogenous" one more similar to the *P. cichorii* mycin system. SyrD (Peptide-3 Exporter Family; TC 3.A.1.113) and Macrolide Exporter Family (MacB)) belong to different superfamilies of ATP-dependent transporters, respectively, ABC1 and ABC3, reflecting their different evolutionary origin (Wang et al. 2009).

6. A plethora of LP-associated LuxR regulators

All the regulators associated with the mycin, peptin, and factin BGCs belong to the LuxR superfamily and dispose of its characteristic carboxyterminal DNA-binding domain. Within LPQ strains but also in some belonging to the *P. asplenii* group, a subset of these LuxR regulators (JesR2, PcoR, and PdfR) are acyl homoserine lactone (AHL) receptors (harbouring the characteristic AHL-binding domain). Their cognate AHL synthase gene *luxI* (i.e. *jesI*, *pcoI*, or *pdfI*), a second regular *luxR* regulator gene, *rfiA*, and a divergent *rhtB* homolog, are also part of this well-conserved "QS cluster" (Figure 3). The following hierarchical model has been described for corpeptin and jessenipeptin production: expression of *rfiA*, together with the AHL synthase gene, is under the control of LuxR; in turn, RfiA activates the expression of CLP biosynthetic and transport genes, resulting in a cell density-dependent CLP production (Licciardello et al. 2007, 2009; Licciardello et al. 2018; Arp et al. 2018). In *Pseudomonas* sp. DF41, a slightly different system occurs, where RfiA but not AHLs are essential for sclerosin production (Berry et al. 2014; Nandi et al. 2016). Even though the corresponding peptin has not yet been characterized, the inactivation of the equivalent *luxR-luxI* gene pair in *Pseudomonas* sp. FW300-N2C3 abolished its pathogenic phenotype (Melnyk et al. 2019). Interestingly, a *luxR*-solo gene (possessing an AHL-binding domain but without a cognate *luxI*) is present downstream of the cichopeptin genes, along with non-adjacent homologs of *salA* and *syrG* regulatory genes (Subramoni and Venturi 2009; Figure 3). RhtB has been previously characterized as a threonine and homoserine transporter possibly involved in AHL export (Zakataeva et al. 1999; Zakataeva et al. 2006). However, a *rhtB* gene is also present in *P. fuscovaginae*/*P. cichorii* and *P. syringae* strains, respectively, linked or unlinked to the RND transporter genes, lacking such QS pair and producing CLPs independently of QS (Quiñones et al. 2005; Mattiuzzo et al. 2011). *P. syringae rhtB*, located downstream of *syrE*, is co-expressed with the *syp*/*syr* genes (Lu et al. 2005). Such prominent conservation within all these BGCs suggests an as yet unexplored functional role.

In *P. syringae*, CLP production is subjected to the control of multiple LuxR regulators. It is not controlled by QS but depends on the GacS/GacA global regulatory system (Quiñones et al. 2005). A complex regulatory cascade composed of, in the order of hierarchy, SalA, SyrG, and SyrF, activates expression of the *syp*/*syr* genes (Wang, Lu, Records, et al. 2006; Wang, Lu, Yang, et al. 2006; Vaughn and Gross 2016). Likewise, the *syrF* homolog, *nunF*, is required for production of both nunamycin and nunapeptin (Hennessy et al. 2017). An homologous regulator, *braD*, is also conserved in the *P. corrugata* group but at a slightly shifted position, downstream the brabantamide operon. The syringafactin BGC is flanked by two additional *luxR* genes (Figure 3), of which only the upstream gene (*syfR*) is strictly required for syringafactin production, at least in *P. syringae* pv. *tomato* DC3000 (Berti et al. 2007). Homologs of both regulators are also associated with the factin BGC of *P. cichorii*, whereas only a *syfR* homolog is retained in *Pseudomonas* sp. QS1027 and *P. asplenii* ES_PA-B8 (Figure 3).

Remarkably, the syringotoxin/fuscopeptin BGC of *P. fuscovaginae* LMG 2158^T lacks regulatory genes (Figure 3). This species contains two QS systems required for virulence but neither regulates CLP production (Mattiuzzo et al. 2011). However, its unlinked BGC for the unknown LP-13 is equipped with four unrelated *luxR* genes, one downstream and three upstream of the NRPS genes.

Phylogenetic analysis of this large set of non-QS LuxR regulators reveals two large clades (Figure 5). The first mainly populated by the peptin-associated RfiA homologs (including JesR1) also accommodates the SalA homologs. The other broad clade encompasses the mycin/brabantamide-associated SyrF homologs, as well as a SyrG branch. The factin-associated LuxR regulators located, respectively, upstream (SyfR) and downstream of the NRPS genes, constitute separate branches in the SyrF-SyrG clade and in the RfiA-SalA clade. Remarkably, the quartette of LP-13 associated LuxR regulators in *P. fuscovaginae*, are also part of these clades but forming separate branches, and it is tempting to speculate that they may play a role in the coordination of CLP production. The occurrence of two LPQ groups is also reflected in the clustering of their LuxRs, in particular for the SyrF/BraD homologs.

7. Biological properties of LPs

7.1. LPs as virulence factors

The CLPs produced by *P. syringae*, *P. fuscovaginae*, *P. corrugata*, and *P. cichorii*, contribute to virulence *via* their phytotoxic activities (Bender et al. 1999; Coraiola et al. 2008; Huang et al. 2015; Strano et al. 2015; Weeraratne et al. 2019). Generally, the amphipathic structure of mycins and peptins allows their insertion into the lipid bilayers of membranes to form pores which induces electrolyte leakage and subsequent death of plant cells (i.e. necrotic symptoms; Hutchison and Gross 1997; Coraiola et al. 2008). However,

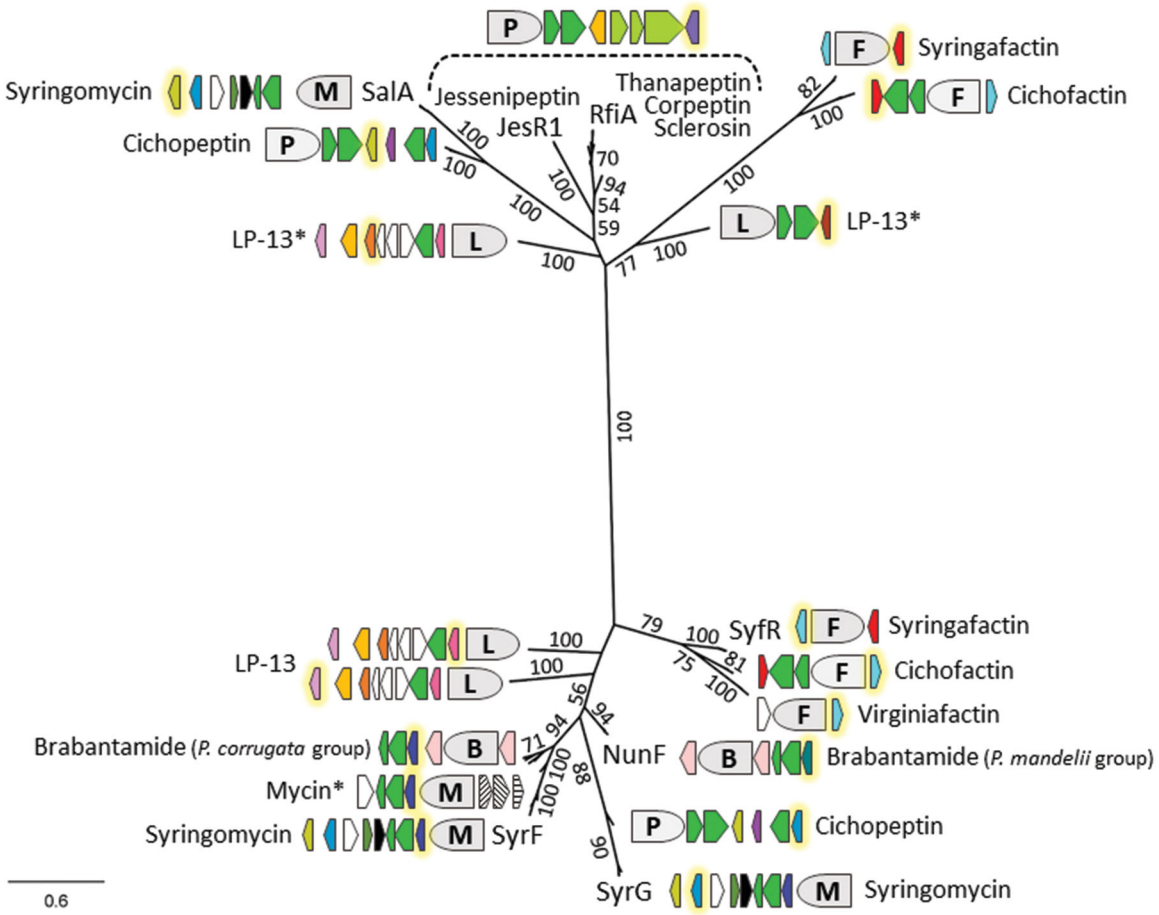

Figure 5. Phylogeny of non-QS LuxR-family proteins associated with mycin, peptin, and factin BGCs. Maximum likelihood phylogenetic tree (PhyML, JTT substitution model) inferred from multiple AA sequence alignment of characterized regulators (JesR1, NunF, RfiA, SalA, SyfR, SyrF, and SyrG) and homologs linked to related BGCs of representative *Pseudomonas* strains. Asterisks indicate LuxRs that are associated with uncharacterized LPs and Mycin* refers to the predicted nunamycin-var1 producers *Pseudomonas* sp. QS1027 and *P. asplenii* ES_PA-B8. The respective regulator genes are shown with a yellow glow and their relative positioning towards the respective NRPS system is indicated (see Figure 3). The syringomycin label includes the sequences retrieved from *P. syringae* pv. *syringae* SM adjacent to its *syrE* remnant. Bootstrap values (percentages of 100 replicates) higher than 50 are shown. The scale bar represents 0.6 substitutions per site.

comparison of the outcome of diverse biological tests reported in the literature shows a trend that CLPs from the mycin family appear more efficient at causing haemolysis (Scaloni et al. 2004; Fiore et al. 2008) while CLPs from the peptin family show stronger phytotoxicity (Ballio et al. 1996; Emanuele et al. 1998; Grgurina et al. 2002; Scaloni et al. 2004). Additionally, their biosurfactant properties seem to have an important role in promoting the spread of the bacteria across the plant (Bender et al. 1999) and syringomycin was shown to contribute (to a greater extent than syringopeptin) to the fitness of *P. syringae* during apoplast colonization (Helmann et al. 2019). Mycins and peptins can work in synergy and altering the production of one or the other induces a strong reduction of virulence (Batoko et al. 1998; Bender et al. 1999; Strano et al. 2015), while in other cases no effect on virulence was observed (Kitten et al. 1998). It was hypothesized that toxin production may contribute differently to virulence depending on the host colonized by *P. syringae* (Bender et al. 1999). Supporting this idea, it was demonstrated that an important factor influencing pore formation is the composition of plant cell membranes, especially the type and abundance of sterols and sphingolipids (Julmanop et al. 1993; Feigin et al. 1997; Bender et al. 1999).

While syringomycin and syringopeptin have a direct phytotoxic activity on plants, syringafactin/cichofactin seems to have a more insidious effect on virulence. Syringafactin represents the major surfactant produced by *P. syringae* pv. *syringae* B728a and it contributes to fitness on leaves under fluctuating humidity (Burch et al. 2014). The activator of flagellar synthesis, FleQ,

Table 3. Antimicrobial activities of LPs.

		Fungi									Oomycetes				Bacteria		
		Ascomycota						*Basidiomycota*							*Firmicutes*	*Proteobacteria*	
LP Family	LP	*B. dothidea*	*B. cinerea*	*G. candidum*	*S. oryzae*	*S. sclerotiorum*	*T. harzianum*	*R. solani*	*R. mucilaginosa*[f]	*S. rolfsii*	*P. aphanidermatum*	*P. capsici*	*P. infestans*	*P. ultimum*	*B. megaterium*	*X. oryzae*	References
Factin	Cichofactin			−					−						−		Pauwelyn et al. (2013); Huang et al. (2015)
Mycin	Brasmycin[a]	+++														−	Zhao et al. (2019)
	Cormycin A														3		Scaloni et al. (2004)
	Nunamycin							+			−						Michelsen, Jensen, et al. (2015)
	Syringomycin E		+++[b]	++[b]	-[b]				+++[b]								Ballio et al. (1996)
			18.75	37.5					6.25						NA[e]		Lavermicocca et al. (1997)
															5		Scaloni et al. (2004)
							+++[c]								+[c]		Fiore et al. (2008)
	Syringotoxin A		25	37.5					6.25						NA[e]		Lavermicocca et al. (1997)
															9		Scaloni et al. (2004)
	Thanamycin		+[d]	−[d]				++[d]		+		−[d]	−[d]	−[d]			Le et al. (2012); Van Der Voort et al. (2015)
Peptin	Fuscopeptin A		++[b]	−[b]	−[b]				+[b]								Ballio et al. (1996)
	Corpeptin A														3.75		Emanuele et al. (1998)
	Cichopeptin			−					−						3.75		Pauwelyn et al. (2013); Huang et al. (2015)
	Braspeptin[a]	+														−	Zhao et al. (2019)
	Nunapeptin							−			+						Michelsen, Jensen, et al. (2015)
	Sclerosin[a]					+									+		Berry et al. (2014)
	Thanapeptin		−[d]	−[d]				−[d]				+[d]	+[d]	+[d]			Van Der Voort et al. (2015)
	Syringopeptin SP22A		+++[b]	−[b]					+[b]								Ballio et al. (1996)
			12.5	37.5					12.5						1.56		Lavermicocca et al. (1997)
				25											6.2		Grgurina et al. (2002); Burch et al. (2014)
									1.95						7.8		Grgurina et al. (2005)
							+[c]								++[c]		Fiore et al. (2008)
	Syringopeptin SP22B								7.8						31.25		Grgurina et al. (2005)
	SP508A								1.95–3.9				−		15.6		Grgurina et al. (2005)
	SP508B								2.7–10.9						31.25		Grgurina et al. (2005)
	SP22PhvA			−											−		Grgurina et al. (2002)
	Peptin-31						+[c]								+[c]		Fiore et al. (2008)
	Syringopeptin SP25A		1.56	NA[e]					37.5						3.12		Lavermicocca et al. (1997)
	SP25 variant			+													Scaloni et al. (1997)

Values correspond to MIC values (μM). Comparison of activities: [a]between mutants and WT, [b]at 20 μM, [c]at 0.5 mg.mL^{-1}, [d]at 50 μM, [e]not active (NA) at 50 μM, [f]formerly *R. rubra*, and *R. piliminae*. *Ascomycota*: *Botryosphaeria dothidea*, *Botrytis cinerea*, *Geotrichum candidum*, *Sarocladium oryzae*, *Sclerotinia sclerotiorum*, *Trichoderma harzianum*. *Basidiomycota*: *Rhizoctonia solani*, *Rhodotorula mucilaginosa*, *Sclerotium rolfsii*. Oomycetes: *Pythium aphanidermatum*, *Phytophthora capsici*, *Phytophthora infestans*, *Pythium ultimum*. Bacteria: *Bacillus megaterium* and *Xanthomonas oryzae*.

exerts a negative control on syringafactin production, apparently suppressing its biosynthesis under conditions favouring swimming motility (Nogales et al. 2015). Inactivation of cichofactin biosynthesis reduces pathogenicity, results in swarming deficiency but enhances biofilm formation (Pauwelyn et al. 2013). Moreover, it was demonstrated that syringafactin facilitates the multiplication of *P. syringae* by interacting with the waxy cuticle of leaves which increases cuticle permeability and allows the diffusion of nutrients out of the tissue (Burch et al. 2014). Perhaps disturbing the plant tissue barrier also facilitates the action of syringomycin and syringopeptin to cause necrosis.

7.2. Anti-microbial activities of LPs

Besides phytotoxicity, CLPs also exhibit a wide anti-microbial activity. This activity probably evolved to ward off microbial competitors that colonize the same niche as CLP producer. Considering the different types of biological tests (with mutants, crude extracts, or pure compounds) on a wide range of microorganisms and in a quite broad range of concentrations, it is clear that, comparison is difficult but it still provides a general view on the different CLP families. Exploring possible biological effects exerted in soil and plant environments, we focus on the biological tests involving microorganisms that are prominent inhabitants of the rhizosphere and/or phyllosphere of plants, particularly excluding human pathogens. Antifungal activity is a common feature but it appears that mycins and peptins show differential activity depending on the type of fungi tested for susceptibility. While it seems that among fungi from the *Ascomycota* branch mycins and 8-ringed peptins act in a species-specific manner, in the *Basidiomycota* branch mycins show a greater antifungal activity then 8-ringed peptins. Conversely, 5-ringed peptins have no or low activity on fungi (*Ascomycota* and *Basidiomycota* branches) but show activity against oomycetes (Table 3). Furthermore, all CLPs exhibit anti-bacterial activity against *B. megaterium* but the MIC values are completely different and dependent on the study and/or the strain of *B. megaterium* used (Ballio et al. 1996; Lavermicocca et al. 1997; Scaloni et al. 2004; Grgurina et al. 2005). Some studies are also reporting anti-bacterial activity against other Gram-positive bacteria, such as *Rhodococcus fascians*, *Micrococcus luteus*, *Bacillus subtilis*, or *Mycobacterium smegmatis* and peptins show higher inhibition then mycins (Lavermicocca et al. 1997; Grgurina et al. 2005; Arp et al. 2018). Jessenipeptin, produced by a grazing-resistant *Pseudomonas*, has amoebicidal activity against the bacterivorous amoeba *Dictyostelium discoideum* and antimicrobial activity against Gram-positive bacteria (Arp et al. 2018).

8. Discussion

In this review, we scrutinized BGC organizations for the production of LPs from the factin (one type), the peptin (five types), and the mycin (four types) families, within a total of 35 *Pseudomonas* genomes (Figures 1 and 2; Table S1). Their MLSA tree is largely congruent with the different types of organization, indicating that the different BGCs have evolved in accordance to the evolutionary history of the *Pseudomonas* species in which they occur. Most of the strains harbour a tight organization of the mycin-peptin genes, forming a chimeric BGC, which indicates a co-evolution of these gene sets (Figure 2). Auxiliary genes for transport and regulation are located in the genomic region between BGCs or positioned tightly downstream of the mycin and peptin NRPS clusters. The transporter genes located at each end are well conserved, even in looser organizations, forming a transporter hug seemly packing up all the required genes (Figure 3). Interestingly, in LPQ strains a brabantamide operon has been integrated within this transporter hug which underlies an important function somehow supporting the action of CLPs.

Three types of peptin BGCs coexist within the *P. syringae* group, designated here as Pep-a and Pep-b configurations, respectively, coding for SP22 and SP25 (and their variants; Table S3) and the Pep-c organization of cichopeptin (Figure 1(C)). The Pep-a type possesses three extra modules compared to its shorter counterpart (Pep-b type), probably resulting from module duplication, while their multiple variants seem to originate mainly from mutations altering A-domain selectivity but keeping the hydrophobic nature of the side chain of AAs (e.g. Val ↔ Leu, Leu ↔ Dhb or Phe ↔ Tyr; Table S3). *P. cichorii* strains and most of the LPQ strains, on the other hand, harbour a completely different peptin-BGC organization (Pep-c type; Figure 1(C)), which suggests that these BGCs have evolved from a common ancestral source, different from the *P. syringae* strains. In *Pseudomonas* sp. Pf153, a shortened version (Pep-e type) is found, apparently obtained by the deletion of nine modules (6–14 from the Pep-c type) and intergenic rearrangement (Figure 1(C) and Table S3). Conceivably, the peptin-BGC of strains from the *P. asplenii* group derive from the SP22-type BGC, with the two first NRPS genes being similar but having a shortened version of the third probably obtained by the

deletion of three modules (i.e. modules 16–18 in SP22; Pep-d type Figure 1(C), Table S3).

Basically, only two main types of mycin BGCs occur: the Myc-a type, with a single multi-domain *SyrE* gene, corresponding to the original syringomycin cluster; and the Myc-b/c types which accommodate two *NRPS* genes, supporting the synthesis of diverse mycins (Figure 1(A,B), Table S2). This diversity of mycins most probably has arisen by gene rearrangement together with mutations altering A-domain selectivity and module shuffling (Table S2). Interestingly, mycin NRPS systems from LPQ strains, *P. asplenii* ATCC 23835^T, *P. fuscovaginae* LMG 2158^T, *P. cichorii* JBC1 and 473, seem to originate from a common ancestor but have further evolved compared to their counterparts in the *P. syringae* group (Myc-b-1 and -2 and Myc-c-1 types; Figures 1(A,B) and 4, Table S2). The Mycin BGCs of LPQ strains are well conserved within each group and bear less diversity. All the mycin producers lack an A-domain in the last module which is complemented by an extra gene (*syrB1* in *P. syringae* with an [A-T] module). However, in some cases, a different version of SyrB1 is observed with an extra T-domain [A-T-T] module (Figure 1(B), Table S2) and such tandem T-domains can increase biosynthetic flux (Zhang et al. 2020). The *syrB1* gene, together with the other *syr* genes, is strictly conserved among all mycin producers which is not surprising considering the fact that this set of genes is responsible for the addition of their characteristic final chlorinated threonine (see Section 2.1).

The factin cluster, on the other hand, when present, is always found apart from this "Peptin-Mycin island" and possesses is own regulatory system (SyfR), which suggests that these clusters were acquired independently. However, in most of the cases it seems that these physically separated clusters have co-evolved long enough for the peptin-type transporter to accommodate the export of LPs from both families (peptin and factin). However, similarly to mono-factin producers of the *P. syringae* complex (Table S1), the cichofactin cluster still harbours cognate transporter genes, indicating that this cluster has been acquired more recently by *P. cichorii*.

P. syringae pv. *syringae* SM highlights an interesting case in which the factin/peptin/mycin BGCs seem to originate from vertical gene transfer (common ancestor with most *P. syringae* strains), but was followed by the acquisition, probably by horizontal gene transfer, of a new mycin BGC (pseudomycin) concomitant with the deletion of the initial mycin BGC (probably syringomycin). Supporting this hypothesis is the reminiscence of the set of conserved genes flanking the initial mycin BGC (Figure 3).

Analysis of LP-BGCs illuminates how mutation and gene rearrangement, closely related to the modular nature of NRPSs, lead to evolutionary diversification. However, to counteract the high selective pressure bearing on such large BGCs, the corresponding metabolites must be essential for the fitness of these bacteria. As elaborated in Section 7, CLPs possess two main functions (phytotoxicity and antimicrobial activity), however, the boundary between phytopathogenic and beneficial (from the human point of view) *Pseudomonas* strains is difficult to delineate.

Within the *P. syringae* complex, most of the analysed strains that are plant pathogenic possess the factin, peptin, and mycin BGCs. However, predicted cichofactin producer strains belonging to the *P. viridiflava* (phylogroup-9) and *P. asturiensis* (phylogroup-7) species lack mycin/peptin BGCs, whereas in phytopathogens within the *P. asplenii* group and *P. fluorescens* complex (*P. corrugata* and *P. mediterranea*) the factin BGC is absent. It was demonstrated that syringafactin is crucial for the interaction with the upper part of plants and its producers appear to mainly cause symptoms aboveground, while most of strains belonging to the *P. fluorescens* complex seem to have more impact on roots or seeds (Höfte and Vos 2007; Melnyk et al. 2019). This might explain why most of the strains within the *P. fluorescens* complex do not feature a factin BGC. Pathogens of the *P. asplenii* group, which cause symptoms on leaves and leaf sheets, harbour a BGC encoding LP-13. This LP may play a role similar to factin-type LPs. However, within the *P. corrugata* group, where the overall BGC organization is well conserved and where CLPs are identical or very similar, some strains behave as phytopathogens (FW300-N2C3, DSM 16733^T, and NCPPB 2445^T) while others are qualified as commensal or biocontrol agent (DF41 and Pf153) (Fuchs 2000; Catara 2007; Berry et al. 2012; Michelsen, Watrous, et al. 2015; Melnyk et al. 2019; Gislason and de Kievit 2020; Yang et al. 2020). In the endophytic metagenome of sugarbeet (Carrión et al. 2019), we identified peptin, mycin, and brabantamide BGC sequences closely matching those of the LPQ island of *P. mediterranea* DSM 16733^T (99.3% nucleotide sequence identity). Peptins seem to show stronger phytotoxicity and one intriguing similarity between some of these potential biocontrol agents is that they may possess a linear version of their peptins (i.e. sclerosin; predicted LP of Pf153). However, phytotoxicity assays are needed to confirm this hypothesis. It also appears that among LPQ strains, CLP production is mostly under the control of QS and thus may occur in a density-dependent manner (Licciardello et al. 2009; Licciardello et al. 2012; Berry

et al. 2014; Arp et al. 2018). The active switch between low and high bacterial density could then explain how they can behave as commensal bacteria or as endophytes and shift to a pathogenic lifestyle when reaching a sufficient number of cells.

Plant signals were demonstrated to modulate CLP production in *P. syringae* (Mo and Gross 1991; Quigley and Gross 1994; Grgurina et al. 1996). In LPQ strains multiple uncharacterized LuxR-type regulators are present (Figures 3 and 5) and it seems possible that some of them also respond to specific secondary metabolites from plants (Subramoni et al. 2011). It also appears that the phytotoxic and antimicrobial activities of CLPs are linked to their ability to interact with membranes, where the nature of the sphingolipids and/or the sterols is of prime importance to determine whether or not a target is sensitive (Balleza et al. 2019). Membrane composition greatly varies according to the stage of development and the different part of the plant (roots, leaves, fruits … .), and it is clear that the pathogenicity of LP-producing *Pseudomonas* is partly driven by the nature of the host but also dependent on the site of interaction. Thus, it is possible that a given *Pseudomonas* strain is pathogenic on above-ground plant parts but neutral or beneficial with biocontrol capacities on plant roots. In the simplest case where a non-sensitive host or part of the host is colonized, CLPs can act as antimicrobial compounds that inhibit or kill competing micro-organisms. If these microbial competitors are pathogenic on economically important plants, the producing *Pseudomonas* strains is considered as a biocontrol agent. It is then not surprising that some *Pseudomonas* species appear ambivalent, behaving on the one hand as a plant pathogen and on the other hand as biocontrol agent (Catara 2007; Melnyk et al. 2019; Gislason and de Kievit 2020). LP phytotoxicity is often assessed with a crude extract or purified compound on *A. thaliana*, tobacco or rice, but one needs to consider that (i) challenging different plant species or tissue (rhizosphere *versus* phyllosphere) may affect outcomes and (ii) the outcome of such assays cannot simply be extrapolated unless *in situ* production of (sufficient amount) LP by the bacteria was previously demonstrated. Finally, LP production is a fine-tuned mechanism under the control of different paths of regulation, and interactions with plants and other micro-organisms allow *Pseudomonas* species to decide whether or not the production of virulence factors, such as LPs, is appropriate to the situation they are in.

9. Perspectives

First, to better understand the fine-tuning of LP production, the function of multiple unknown LuxR-family regulators has to be characterized, particularly with respect to the chemical nature and origin of effector molecules that activate them (plant, microbial, or other environmental source). A subset of these regulators are part of a QS system controlling LP production and AHLs secreted by other bacteria occupying a common niche, pseudomonads as well as other AHL-producing bacteria, may influence LP production. Such QS cross-talk has been inferred from phenotypic complementation of LPQ-associated phytopathogenicity in a gnotobiotic *Arabidopsis* system upon co-inoculation with a non-virulent QS mutant and a different non-pathogenic LPQ strain (Melnyk et al. 2019). Cooperative phytopathogenicity driven by commensal AHL sharing has been observed for the olive tree pathogen *P. savastanoi* (Hosni et al. 2011; Caballo-Ponce et al. 2018). Conceivably, such signal sharing may also support cooperative microbial antagonism contributing to biocontrol (Besset-Manzoni et al. 2018). On the other hand, many plant-associated micro-organisms are also able to interfere with such interactions between community members by Quorum Quenching (QQ) mechanisms (Chan et al. 2011; Jafra et al. 2006). It will be of interest to determine to which extent interactions with other plant-associated micro-organisms can modulate LP production and, consequently, pathogenicity, and/or antimicrobial activity of the producing *Pseudomonas* strain.

Second, the inspection of *Pseudomonas* genomes allowed us to predict the peptide sequence of multiple new variants and to identify potential producers of known CLPs (e.g. syringotoxin, syringostatin, or syringopeptin SP508). *P. cichorii* is predicted to synthesize pseudomycin, in accordance with the molecular mass of a metabolite co-produced with cichofactin and cichopeptin (Pauwelyn 2012). Additional predictions awaiting validation comprise a novel variant of SP22 and cichopeptin, three non-described analogues of syringopeptin SP25 and additional LPQ island-derived peptins (Tables S2 and S3). Furthermore, new mycin variants are also awaiting characterization: strain DF41 strain likely produces a thanamycin peptide variant; although our peptide sequence prediction for brasmycin (strain 11K1) matches the one for thanamycin, the molecular masses are different (Michelsen, Jensen, et al. 2015; Zhao et al. 2019); and strain QS1027 is a candidate nunamycin analogue producer not affiliated with the *P. mandelii* LPQ-group.

Additionally, co-occurrence of a number of cryptic BGCs (NRPS and NRPS-PKS) was highlighted. The

corresponding metabolites (LP-8, LP-13, rimosamide-related molecule) await chemical and functional characterization, including possible synergy with (other) LPs for phytopathogenic and/or antimicrobial activities. Rimosamide was shown to antagonize the antimicrobial activity of the *Streptomyces griseochromogenes* blasticidin against *Bacillus cereus* (McClure et al. 2016). On the other hand, Arp et al. (2018) have demonstrated the synergistic activity of jessenipeptin and the antibiotic mupirocin towards *S. aureus* but the fact that each BGC possesses its own associated QS genes indicates that the production of each metabolite can (also) be regulated separately (Thomas et al. 2010). The brabantamide genes were shown to be co-transcribed with CLP BGCs and their clustering with CLP-associated transporter genes hints to potential co-secretion of brabantamide and CLPs by LPQ strains (Van Der Voort et al. 2015). Notably, in the study of Melnyk et al. (2019), the brabantamide operon was co-deleted with CLP genes and, therefore, not individually accounted for the assessment of FW300-N2C3 mutant phenotypes regarding phytopathogenicity. Brabantamide is a glycosylated LP with phospholipase inhibitory activity and was shown to have a much broader antimicrobial activity than syringomycin, inhibiting a broad range of Gram-positive bacteria (Busby et al. 2000; Thirkettle et al. 2000; Reder-Christ et al. 2012). When considering the functions of these associated metabolites it is tempting to speculate that they can potentially contribute to antimicrobial or phytopathogenic behaviour.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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